ORIGINAL CONTRIBUTION

Exhibit 1

# Stem Cell Mobilization by Granulocyte Colony-Stimulating Factor in Patients With Acute Myocardial Infarction

## A Randomized Controlled Trial

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**Context** Experimental studies and early phase clinical trials suggest that transplantation of blood-derived or bone marrow–derived stem cells may improve cardiac regeneration and neovascularization after acute myocardial infarction. Granulocyte colony-stimulating factor (G-CSF) induces mobilization of bone marrow stem cells.

**Objective** To assess the value of stem cell mobilization by G-CSF therapy in patients with acute myocardial infarction.

**Design, Setting, and Patients** Randomized, double-blind, placebo-controlled trial of patients diagnosed with ST-segment elevation acute myocardial infarction who had successful reperfusion by percutaneous coronary intervention within 12 hours after onset of symptoms in Germany between February 24, 2004, and February 2, 2005.

**Interventions** Patients were randomly assigned to receive subcutaneously either a daily dose of 10 μg/kg of G-CSF or placebo for 5 days.

**Main Outcome Measures** The primary end point was reduction of left ventricular infarct size according to technetium Tc 99m sestamibi scintigraphy performed at baseline and at 4 to 6 months after randomization. Secondary end points included improvement of left ventricular ejection fraction measured by magnetic resonance imaging and the incidence of angiographic restenosis.

**Results** Of the 114 patients, 56 were assigned to receive treatment with G-CSF and 58 were assigned to receive placebo. Treatment with G-CSF produced a significant mobilization of stem cells. Between baseline and follow-up, left ventricular infarct size according to scintigraphy was reduced by a mean (SD) of 6.2% (9.1%) in the G-CSF group and 4.9% (8.9%) in the placebo group ($P=.56$) and left ventricular ejection fraction was improved by 0.5% (3.8%) in the G-CSF group and 2.0% (4.9%) in the placebo group ($P=.14$). Angiographic restenosis occurred in 19 (35.2%) of 54 patients in the G-CSF group and in 17 (30.9%) of 55 patients in the placebo group ($P=.79$). The most common adverse event among patients assigned to G-CSF was mild to moderate bone pain and muscle discomfort.

**Conclusion** Stem cell mobilization by G-CSF therapy in patients with acute myocardial infarction and successful mechanical reperfusion has no influence on infarct size, left ventricular function, or coronary restenosis.

**Clinical Trial Registration** ClinicalTrials.gov Identifier: NCT00126100

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ACUTE MYOCARDIAL INFARCTION (AMI) is followed by enhanced spontaneous mobilization of bone marrow–derived stem cells.[1-3] The extent and duration of this mobilization have been reported to correlate with improvement of left ventricular function.[1,4] These findings support the hypothesis that enhancing mobilization of endogenous stem cells may have favorable effects on left ventricular recovery after an AMI.

Granulocyte colony-stimulating factor (G-CSF) is an effective stimulus for

**See also p 1058 and Patient Page.**

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mobilization of bone marrow–derived stem cells into the peripheral circulation. Several studies have shown that G-CSF–mobilized stem cells are recruited to ischemic myocardium and differentiate into specialized cells such as cardiomyocytes, endothelial cells, and smooth muscle cells.[5-9] They also may accelerate the healing process by induction of matrix metalloproteinases and vascular endothelial growth factor.[10,11] In addition to mobilization of bone marrow–derived stem cells, G-CSF is known to induce proliferation and enhance survival of cardiomyocytes through activation of specific G-CSF receptors within the heart.[12] Other experimental studies have shown attenuated ventricular expansion in association with increased transforming growth factor β expression and collagen expression in the infarcted area after G-CSF administration.[13] Thus, G-CSF may regenerate myocardium by mobilizing bone marrow–derived stem cells, by direct effects on cardiomyocytes, or by the release of proangiogenic mediators.

Recently, 3 clinical trials[14-16] investigated the safety and feasibility of stem cell mobilization by G-CSF in 11, 20, and 50 patients with AMI, respectively. All studies showed improvement of left ventricular function in the groups treated with G-CSF. However, there was an increased risk of restenosis in 1 trial.[14] Due to the limited number of patients enrolled,[14-16] it is difficult to define the role of G-CSF treatment in patients with AMI after successful revascularization at this stage. Therefore, the purpose of this randomized, double-blind, placebo-controlled study was to assess the value of G-CSF treatment in a larger cohort of patients with AMI.

## METHODS

Patients were enrolled in the Regenerate Vital Myocardium by Vigorous Activation of Bone Marrow Stem Cells (REVIVAL-2) trial 5 days after an ST-segment elevation AMI between February 24, 2004, and February 2, 2005, at the Deutsches Herzzentrum and at the First Medizinische Klinik, Klinikum rechts der Isar, both in Munich, Germany. The diagnosis of AMI was established by the presence of chest pain lasting 20 minutes or longer, an ST-segment elevation of 0.1 mV or greater in 2 or more limb leads, an ST-segment elevation of 0.2 mV or greater in 2 or more contiguous precordial leads, or left bundle-branch block of presumably new onset on surface electrocardiogram. To be included in the study, patients were required to have had successful reperfusion by percutaneous coronary intervention (performed ≤12 hours from symptom onset) and an infarct size of at least 5% of the left ventricle in single-photon emission computed tomography with technetium Tc 99m sestamibi (performed before randomization). Exclusion criteria were age younger than 18 years or older than 80 years, congestive heart failure defined as Killip class higher than II, electrical or hemodynamic instability, a history of prior myocardial infarction, autoimmune diseases, fructose intolerance, malignancies, incompatibility of G-CSF, and known or suspected pregnancy. The study protocol was approved by the institutional ethics committee responsible for both participating centers. Each participant provided written informed consent.

Patients were randomly assigned 5 days after an AMI to receive subcutaneously either a daily dose of 10 μg/kg of G-CSF (Neupogen, Amgen, Thousand Oaks, Calif) or placebo for 5 days. A computer-generated randomization sequence with a block size of 10 and no stratification was used; results were kept in sealed envelopes. The physicians who enrolled the patients were unaware of the randomization sequence and the block size. Double-blinding was achieved by the use of similar-appearing injection syringes containing either G-CSF or placebo.

### Blood Analyses

$CD34^+$ cells were quantified at days 1, 3, 5, and 7 after randomization. The analysis of peripheral blood $CD34^+$ cells was performed from heparinized blood samples. Quantification was performed using TrueCount beads, APC-anti-CD45, FITC-anti-CD34, and PE-anti-CD133 using an FACS Calibur (Becton Dickinson, Lexington, Ky) according to standardized procedures.[17] The absolute number of $CD34^+$ cells was obtained from the absolute $CD45^+$ cell count and the percentage of $CD34^+$ cells.

Serum creatine kinase and its MB isoenzyme, lactate dehydrogenase, alkaline phosphatase, C-reactive protein concentrations, and differential blood count were determined in the clinical chemistry laboratory from blood samples taken before and daily after randomization as well as at follow-up.

### Technetium Tc 99m Sestamibi Single-Photon Emission Computed Tomography

Technetium Tc 99m sestamibi single-photon emission computed tomography was performed at baseline and at 4 to 6 months after randomization for each patient at rest. Detailed descriptions of the methods used to measure left ventricular infarct size have been published.[18-20] All studies were processed and evaluated in the scintigraphic core laboratory by experienced operators who were blinded to the assigned therapy.

### Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) was performed before and at 4 to 6 months after randomization in patients in the supine position with a 1.5-T clinical scanner (Siemens Sonata, Erlangen, Germany) equipped with a dedicated cardiac-phased array surface coil. All images were obtained during repeated periods of breath holding and were gated to the electrocardiogram. Cine images of the entire left ventricle were acquired in contiguous short-axis views using a gradient-echo MRI sequence with a slice thickness of 8 mm. To determine the left ventricular ejection fraction (LVEF), an observer outlined the left ventricular borders on the short-

axis cine images. The LVEF was calculated by subtracting the volume at end systole from the volume at end diastole divided by the volume at end diastole. All studies were processed and evaluated at the MRI core laboratory by experienced operators who were blinded to the assigned therapy.

### Angiographic Evaluation

We used a nonionic contrast medium (Imeron, Altana, Germany) in all patients. Left ventricular and coronary angiograms were assessed at the angiographic core laboratory by personnel blinded to treatment group allocation. Quantitative assessment of angiograms was performed with the use of an automated edge detection system (CMS Version 6.0.10.0, Medis Medical Imaging Systems, Nuenen, the Netherlands). The LVEF was determined by the area-length method. We used the centerline method to quantify regional left ventricular wall motion by measuring the number of chords within the region of interest showing hypokinesis.[21] Coronary angiographic sequences were preceded by an intracoronary injection of nitroglycerin. The analysis segment comprised the stented segment and the 5-mm proximal and distal edges of stent(s).

### Follow-up Protocol

In-hospital follow-up protocol consisted of serial electrocardiographic recordings and determinations of several laboratory measurements (see prior subsections of the "Methods" section). Patients usually stayed in the hospital for 2 to 3 days after completion of the study treatment to monitor for potential adverse effects. Patients were contacted by telephone at 30 days after enrollment in the study. All patients were asked to return at 4 to 6 months after randomization for a clinical checkup and follow-up scintigraphy, MRI, and angiography.

### Definitions and Study End Points

The primary end point was the reduction of infarct size measured as the difference in left ventricular infarct size at baseline (study entry) and follow-up by single-photon emission computed tomography. Secondary end points were improvement in LVEF from baseline to follow-up by MRI as well as angiographic restenosis defined as a diameter stenosis of 50% or greater by follow-up angiography. Other measures assessed were left ventricular volumes by MRI, LVEF, and number of hypokinetic chords by angiography. We also monitored for the occurrence of the following major adverse cardiac events: death, recurrent myocardial infarction, and reintervention in the infarct-related artery. Diagnosis of recurrent infarction was based on the presence of at least 2 of the following criteria: new ST-segment changes and an increase in creatine kinase and creatine kinase-MB of at least 50% more than the previous level in at least 2 samples reaching 3 times or greater the upper limit of normal.

### Statistical Analyses

Sample size calculation was based on the following assumptions regarding the primary end point of the trial: a left ventricular infarct size reduction of at least 6% higher in the G-CSF group than in the placebo group with a common SD of the difference of 10%. This difference represents one third of the infarct size reduction achieved with mechanical reperfusion.[19] Accordingly, a total sample size of 90 patients with paired scintigraphic studies was required to detect this difference with a power of 80% and a 2-sided $\alpha$ error of .05. The overall number of patients enrolled was expanded to 114 to accommodate for possible missing scintigraphic studies.

All analyses were performed on the basis of the intention-to-treat principle using data from all patients as randomized. Categorical data are presented as counts or proportions (percentages). Continuous data are presented as mean (SD). Differences between the groups were assessed using the Fisher exact test for categorical data and the nonparametric Wilcoxon rank sum test for continuous data. A 2-tailed $P<.05$ was considered to indicate statistical significance. We used S-Plus version 4.5 (S-PLUS, Insightful Corp, Seattle, Wash) for the statistical analyses.

## RESULTS

The flow of the participants through the REVIVAL-2 trial appears in the FIGURE. Baseline characteristics of the patients appear in TABLE 1. All patients had a successful percutaneous coronary intervention 5 days before randomization with implantation of bare metal stents in 51 of the 56 patients in the G-CSF group and 50 of the 58 patients in the placebo group. Drug-eluting stents were implanted in the remaining patients.

In the G-CSF group, 15 patients (27%) had complaints during the treatment: mild to moderate bone pain and muscle discomfort in 7 patients, tiredness in 3 patients, mild fever in 2 patients, exanthema in 2 patients, and nausea in 1 patient. In the placebo group, 6 patients (10%) had complaints during the treatment: mild muscle discomfort in 1 patient, fever in 1 patient, tiredness and headache in 2 patients, exanthema in 1 patient, and nausea in 1 patient.

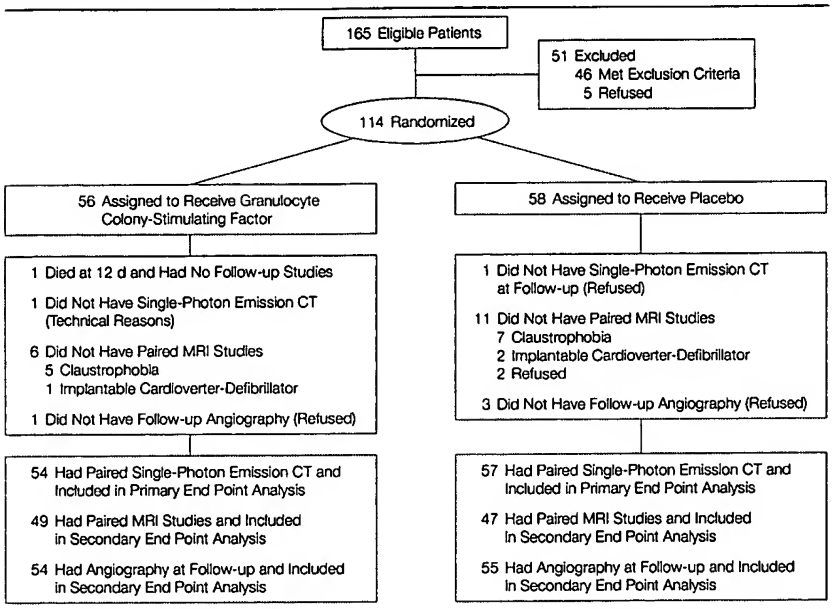
The change in laboratory measures due to study treatment is shown in TABLE 2. In the G-CSF group, both $CD34^+$ and white blood cells gradually increased, peaking at day 5 after initiation of therapy. Additionally, platelet count decreased at the end of the treatment period. The G-CSF group showed an increase in lactate dehydrogenase, alkaline phosphatase, and C-reactive protein. At follow-up, all these measures had returned to baseline values. There was no effect of G-CSF on the level of creatine kinase-MB during the treatment period (Table 2).

During the follow-up period, 1 patient (1.8%) in the G-CSF group died of ventricular fibrillation 12 days after enrollment in the study and 1 patient (1.7%) in the placebo group developed a nonfatal myocardial reinfarction.

### Infarct Size

The results of single-photon emission computed tomography regarding infarct size at baseline and follow-up appear in TABLE 3. Paired scintigraphic examinations were missing in 2 patients in the G-CSF group and in 1 patient in the placebo group. There were no differences in infarct size between the 2 groups. The mean (SD) reduction of left ventricular infarct size from baseline to follow-up was 6.2% (9.1%) in the G-CSF group and 4.9% (8.9%) in the placebo group ($P=.56$).

**Figure.** Flow of REVIVAL-2 Study Participants




CT indicates computed tomography; MRI, magnetic resonance imaging; REVIVAL-2, Regenerate Vital Myocardium by Vigorous Activation of Bone Marrow Stem Cells.

**Table 1.** Baseline Characteristics*

| | G-CSF (n = 56) | Placebo (n = 58) | *P* Value |
|---|---|---|---|
| Age, mean (SD) y | 59.4 (12.0) | 59.8 (10.3) | .93 |
| Women | 12 (21.4) | 12 (20.7) | .91 |
| Body mass index, mean (SD)† | 27.2 (3.6) | 27.5 (4.1) | .98 |
| Arterial hypertension | 31 (55.4) | 41 (70.7) | .13 |
| Diabetes | 6 (10.7) | 10 (17.2) | .46 |
| Current smoker | 25 (44.6) | 27 (46.6) | .99 |
| Hypercholesterolemia | 23 (41.1) | 24 (41.4) | .89 |
| Multivessel disease | 31 (55.4) | 38 (65.5) | .36 |
| Infarct localization | | | |
| Anterior | 30 (53.6) | 28 (48.3) | .71 |
| Inferior | 19 (33.9) | 18 (31.0) | .90 |
| Lateral | 7 (12.5) | 12 (20.7) | .36 |
| Heart rate, mean (SD), beats/min | 68 (9) | 68 (10) | .81 |
| Blood pressure, mean (SD), mm Hg | | | |
| Systolic | 110 (15) | 109 (15) | .86 |
| Diastolic | 66 (9) | 66 (8) | .28 |
| Concomitant therapy at discharge | | | |
| β-Blockers | 56 (100) | 57 (98.3) | .98 |
| ACE inhibitors | 55 (98.2) | 57 (98.3) | .97 |
| Statins | 55 (98.2) | 56 (96.6) | .97 |
| Nitrates | 0 | 2 (3.4) | .51 |

Abbreviations: ACE, angiotensin-converting enzyme; G-CSF, granulocyte colony-stimulating factor.
*Data are presented as number (percentage) unless otherwise indicated.
†Calculated as weight in kilograms divided by the square of height in meters.

### Left Ventricular Function

The results of MRI regarding left ventricular volume and LVEF measurements at baseline and follow-up also appear in Table 3. There were no differences regarding left ventricular volumes and LVEF. The mean (SD) improvement of LVEF from baseline to follow-up was 0.5% (3.8%) in the G-CSF group and 2.0% (4.9%) in the placebo group ($P=.14$). This improvement was significant only in the placebo group ($P<.001$ vs $P=.17$ in the G-CSF group). Moreover, the reduction in left ventricular end-systolic volume index was only significant in the placebo group ($P=.03$ vs $P=.41$ in the G-CSF group).

There was no interaction between treatment effect and left ventricular function at baseline. More specifically, in the lower quartile of left ventricular function (baseline LVEF ≤45.0%), the mean (SD) improvement in LVEF from baseline to follow-up was 1.1% (3.3%) in the G-CSF group and 3.2% (7.4%) in the placebo group ($P=.25$). In the upper quartile of left ventricular function (baseline LVEF >55.4%), LVEF had a mean (SD) change from baseline to follow-up by −1.5% (3.5%) in the G-CSF group and 0.1% (3.2%) in the placebo group ($P=.25$).

Additionally, the results of angiography performed at follow-up appear in Table 3. There were no significant differences regarding both global and regional left ventricular function between the 2 groups.

### Restenosis

The slight difference in the incidence of angiographic restenosis (Table 3) disappeared when the analysis was confined to patients treated with bare metal stents. Among patients with bare metal stents and follow-up angiography, restenosis was observed in 18 (36.0%) of

the 50 patients in the G-CSF group and in 17 (36.2%) of the 47 patients in the placebo group ($P=.87$). Reintervention in the infarct-related vessel was required in 16 (28.6%) of the 56 patients in the G-CSF group and in 18 (31.0%) of the 58 patients in the placebo group ($P=.93$).

## COMMENT

There is increasing evidence that stem cells contribute to regeneration of cardiac tissue as a natural healing process following AMI, thus opening up new prospects for stem-cell based therapies.[6,7,22-26] Our expectations regarding the impact of G-CSF therapy on infarct size and left ventricular function in patients with AMI after successful revascularization were based on the positive findings of experimental and early phase clinical studies.[5,9,10,12,14-16,27] The present randomized, double-blind, placebo-controlled trial addressed the impact of bone-marrow stem cell mobilization by G-CSF for myocardial regeneration in patients with AMI successfully treated with primary percutaneous coronary intervention. Granulocyte colony-stimulating factor is widely used to accelerate restoration of neutrophil count after chemotherapy or bone marrow transplantation. Consistent with previous studies,[14-16,28] the effectiveness of the treatment in patients with AMI was shown by a marked increase in circulating $CD34^+$ cells as well as granulocytes, monocytes, and lymphocytes. However, our trial demonstrated that effective stem cell mobilization with G-CSF does not alter infarct size or left ventricular function after AMI. Moreover, in contrast to other studies,[14,29] no increase in the risk of restenosis or major adverse cardiac events was observed with G-CSF treatment.

The LVEF evaluated 5 days after AMI in the present study was comparable with that observed in patients with AMI treated by stem cell therapy in previous studies.[14-16,30-33] Six months after AMI, infarct size significantly decreased in both the G-CSF group and the placebo group accompanied by a decrease in left ventricular end systolic

**Table 2.** Laboratory Data

| | | Mean (SD) Level, by Day | | | | | |
|---|---|---|---|---|---|---|---|
| | Normal Range | 0 | 1 | 3 | 5 | 7 | Follow-up |
| $CD34^+$ cells, /μL | | | | | | | |
| G-CSF | 0-5 ×10⁶/L | | 12 (17) | 44 (167)* | 72 (154)* | 59 (148)* | |
| Placebo | | | 9 (22) | 6 (11) | 5 (6) | 7 (12) | |
| White blood cells, ×10⁹/L | 4-9 ×10⁹/L | | | | | | |
| G-CSF | | 8 (2) | 26 (8)* | 42 (14)* | 48 (15)* | 19 (7)* | 7 (2) |
| Placebo | | 8 (2) | 9 (6) | 9 (10) | 8 (6) | 8 (2) | 7 (2) |
| Neutrophils, ×10⁹/L | 1.8-7.3 ×10⁹/L | | | | | | |
| G-CSF | | | 22.2 (6.2)* | 34.0 (11.0)* | 33.2 (11.3)* | 13.4 (16.5)* | 4.0 (1.7) |
| Placebo | | | 5.2 (2.8) | 5.1 (3.8) | 4.9 (3.8) | 4.6 (1.8) | 4.1 (1.6) |
| Lymphocytes, ×10⁹/L | 1.0-4.8 ×10⁹/L | | | | | | |
| G-CSF | | | 2.0 (1.2) | 3.5 (1.8)* | 4.4 (2.2)* | 2.3 (1.1)* | 1.8 (0.6) |
| Placebo | | | 1.8 (1.0) | 1.8 (0.6) | 1.9 (0.7) | 1.9 (0.6) | 1.7 (0.8) |
| Monocytes, ×10⁹/L | 0.07-0.84 ×10⁹/L | | | | | | |
| G-CSF | | | 1.4 (0.7)* | 1.8 (0.9)* | 2.9 (1.9)* | 1.2 (0.6)* | 0.6 (0.2) |
| Placebo | | | 0.7 (0.3) | 0.6 (0.3) | 0.7 (0.5) | 0.6 (0.3) | 0.5 (0.2) |
| Platelets, ×10⁹/L | 130-370 ×10⁹/L | | | | | | |
| G-CSF | | 256 (78) | 262 (74) | 281 (86) | 287 (99) | 237 (99)* | 233 (58) |
| Placebo | | 241 (59) | 258 (70) | 278 (85) | 295 (78) | 305 (98) | 231 (59) |
| Creatine kinase-MB, U/L | <24U/L | | | | | | |
| G-CSF | | 20 (5) | 15 (4) | 14 (4) | 14 (5) | 14 (4) | 14 (4) |
| Placebo | | 21 (7) | 15 (5) | 14 (7) | 14 (5) | 13 (4) | 13 (4) |
| Lactate dehydrogenase, U/L | 135-370 U/L | | | | | | |
| G-CSF | | 465 (201) | 435 (206)* | 467 (173)* | 604 (245)* | 406 (162)* | 192 (37) |
| Placebo | | 475 (194) | 368 (121) | 311 (91) | 284 (75) | 260 (57) | 196 (63) |
| Alkaline phosphatase, U/L | 60-130 U/L | | | | | | |
| G-CSF | | 90 (25) | 85 (25) | 146 (34)* | 235 (62)* | 188 (43)* | 79 (37) |
| Placebo | | 97 (54) | 84 (36) | 90 (42) | 95 (48) | 93 (43) | 76 (24) |
| C-reactive protein, mg/L | 0-5 mg/L | | | | | | |
| G-CSF | | 3.4 (3.0) | 2.7 (3.3) | 2.7 (3.6) | 2.4 (3.1)* | 2.3 (4.1)* | 0.4 (0.5) |
| Placebo | | 4.3 (3.0) | 2.7 (2.8) | 2.0 (2.0) | 1.8 (2.8) | 1.5 (2.2) | 0.5 (0.8) |

Abbreviation: G-CSF, granulocyte colony-stimulating factor.
*$P<.05$ for G-CSF group vs placebo group.

**Table 3.** Quantitative Data From Scintigraphy, Magnetic Resonance Imaging, and Angiography

| | G-CSF (n = 56) | Placebo (n = 58) | *P* Value |
|---|---|---|---|
| Technetium Tc 99m sestamibi scintigraphy, No. (%) | 54 (96) | 57 (98) | |
| LV infarct size, mean (SD), % | | | |
| At baseline | 19.1 (15.3) | 19.1 (17.6) | .53 |
| At follow-up | 12.9 (15.6) | 14.2 (17.4) | .78 |
| Magnetic resonance imaging, No. (%) | 49 (88) | 47 (81) | |
| Baseline | | | |
| LV end volume index, mean (SD), mL/m$^2$ | | | |
| Diastolic | 93.3 (18.7) | 89.6 (17.5) | .12 |
| Systolic | 46.1 (13.7) | 46.1 (15.2) | .61 |
| LV ejection fraction, mean (SD), % | 51.3 (8.2) | 49.2 (8.7) | .33 |
| Follow-up | | | |
| LV end volume index, mean (SD), mL/m$^2$ | | | |
| Diastolic | 92.4 (20.5) | 87.8 (21.9) | .16 |
| Systolic | 45.5 (15.5) | 43.7 (17.5) | .27 |
| LV ejection fraction, mean (SD), % | 51.8 (7.7) | 51.2 (9.0) | .85 |
| Angiography (follow-up), No. (%) | 54 (96) | 55 (95) | |
| LV ejection fraction, mean (SD), % | 56.6 (9.6) | 56.0 (11.3) | .76 |
| Hypokinetic chords, mean (SD) | 18.6 (16.8) | 18.8 (18.2) | .80 |
| Restenosis, No. (%) | 19 (35.2) | 17 (30.9) | .79 |

Abbreviations: G-CSF, granulocyte colony-stimulating factor; LV, left ventricular.

volumes and slight improvement in LVEF. Therefore, our data indicate that functional recovery after AMI occurs even later than 5 days after successful reperfusion independent of stem cell therapy. Comparable changes in left ventricular function have been described in the treatment group but not in the control group of earlier clinical studies investigating the effect of stem cell mobilization by G-CSF[15,16] or of intracoronary transplantation of G-CSF mobilized stem cells.[14] In accordance with our findings, Valgimigli et al[15] have described improvement of left ventricular function in both a G-CSF group and in a control group. The improvement of left ventricular function is the result of the recovery of the stunned myocardium after successful reperfusion[34-37] and of optimized drug therapy after AMI.

The negative finding of the current study has several possible explanations. Mobilized stem cells might not have homed to the infarcted myocardium. Homing and engraftment of mobilized stem cells into the site of myocardial injury displays an important step in stem cell–based regeneration of the injured myocardium.[22,23] For example, the chemokine SDF-1 plays a crucial role but is not by itself sufficient for the recruitment of circulating stem cells to the site of cardiac ischemia.[38] Therefore, the concerted expression and activation of several genes is a prerequisite for successful recruitment of mobilized stem cells.

It is possible that at the time of stem cell mobilization in our study, the milieu of the infarcted myocardium did not allow significant recruitment of stem cells. In previous experimental studies reporting positive effects of G-CSF treatment in AMI,[5,12,16,27] G-CSF therapy was started before or right after an AMI. Animal data suggest that stem cell homing induced by expression of SDF-1 only takes place before day 7 after an AMI.[38,39] However, CD34$^+$ stem cell mobilization occurs naturally in patients with an AMI peaking at day 7.[40,41] Moreover, results from the REPAIR-AMI trial[42] showed that improvement of left ventricular function correlated with the time in which intracoronary stem cell transplantation was performed. The beneficial effects were most prominent in patients who received the stem cell transplantation more than 5 days after an AMI and there was no improvement in left ventricular function in patients who received treatment in 4 days or less after an AMI.[42] These data indicate that myocardial stem cell homing may still be improved more than 5 days after an AMI.

The expression of the CD34 surface antigen is found on hematopoietic progenitor cells, endothelial progenitor cells, and mature endothelial cells.[43] Granulocyte colony-stimulating factor mobilizes mainly hematopoietic progenitor cells.[28] It is conceivable that hematopoietic and endothelial progenitor cells may play different roles in tissue repair (ie, while endothelial progenitor cells induce angiogenesis, hematopoietic progenitor cells generate new cardiomyocytes). Recently, the concept of transdifferentiation from hematopoietic progenitor cells to cardiomyocytes has been challenged[44,45] and the role of endothelial progenitor cells as a source of proangiogenic cytokines for the ischemic myocardium has been favored.[46]

The functional activity of stem cells mobilized by treatment with G-CSF might have been compromised due to the release of immature stem cells with limited capacity of homing to ischemic myocardium.[47,48] Finally, we cannot be sure that G-CSF itself does not have a negative impact on cardiac regeneration after AMI, although treatment with G-CSF has inhibited apoptosis and improved survival of cardiomyocytes after AMI in mice.[12]

Previous clinical trials suggested a positive impact of G-CSF–induced stem cell mobilization on left ventricular function after AMI.[14-16] The largest study by Ince et al[16] included 50 patients with AMI and was an open-label study without a placebo group. The other 2 studies also lacked a double-blind design and the follow-up results were available from only 14 and 11 patients, respectively.[14,15] Therefore, the present study represents the first randomized, double-blind, placebo-controlled trial on the value of G-CSF–induced stem cell mobilization in patients with AMI. The REVIVAL-2 trial had a cohort that

was larger than all 3 previous trials taken together[14-16] and had a relatively long follow-up period based on sensitive assessment methods of left ventricular function and infarct size.

In conclusion, use of G-CSF therapy to mobilize bone marrow–derived stem cell does not improve left ventricular recovery in patients with AMI after successful mechanical reperfusion.

**Author Contributions:** Dr A. Schömig had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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There is one psychological peculiarity in the human being that always strikes one: to shun even the slightest signs of trouble on the outer edge of your existence at times of well-being . . . to try not to know about the sufferings of others and your own or one's own future sufferings, to yield in many situations, even important spiritual and central ones—as long as it prolong's one's well-being.

—Alexander I. Solzhenitzyn (1918- )